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IN ADVANCE BY FACSIMILE
ORIGINAL TO FOLLOW

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In Zusammenarbeit mit
Rechtsanwälten
Dr. Wolfram Stadtler
Stephan Biagosch

4. November 2003

File No.: PCT/EP02/14511
Applicant: Petzelt
Our Ref: P 3098 - sch / tz

In Response to the Written Opinion (PCT Rule 66) of August 5, 2003:

1. Requests

The applicant requests a detailed substantive examination.

Further, a second written opinion is requested, if the Examiner does not agree that the claims meet the criteria set forth in Article 33 (2-4) PCT.

2. Reply to the Written Opinion

The claims refer to a modified cyplasin without a secretory signal sequence. The applicant has found out that deleting the secretory signal sequence from the protein does not impair the cytotoxic activity of cyplasin.

The GENEMBL-data base extract "Aplysia punctata mRNA for cyplasin L (ek431 gene)" discloses a nucleotide sequence of the native cyplasin. The nucleic

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acid molecule specified in claim 1 is not derivable from that document. In particular, it is not derivable from said extract which part of the sequence causes the cytotoxic acitivity of cyplasin. Thus, the claims are new and inventive over that reference.

Petzelt et al. (Cell Biology International vol. 25, no. 2, 2001, page A23) describe the cytotoxic activity of native cyplasin. That document does not disclose a protein which is encoded by the sequence specified in claim 1. Further that document does neither disclose a process for making a recombinant modified cyplasin nor a pharmaceutical composition comprising said protein. Thus, the claims are new and inventive over that reference.

Suzuki et al. (Protein Engineering vol. 13, no. 2, pp. 73-76, 2000) describe that the deletion of the signal sequence resulted in complete loss of the lethal acivity of the yeast killer toxin SMKT (p. 74, right column, 3rd paragraph). Further, the authors refer to Tokunaga et al. (Nucleic Acids Res. 1989; 17 (9): pp. 3435-46) who describe that the expression of the *K. lactis* toxin without a signal sequence is lethal for the host cells from inside, while the killer subunit of said toxin alone does not kill the sensitive cells from outside (see the enclosed abstract). On the contrary, the claimed modified protein is not toxic for the host cells from inside, but kills the sensitive cells from outside. Hence, the teaching of Suzuki et al. does not anticipate the present application.

In summary, none of the above references cited in the International Search Report - either alone or in combination - suggests the claimed specific modified cyplasin without a signal sequence to a person skilled in the art. Thus, the claims fulfil the requirements set forth in Article 33 PCT.

Patent-Attorney
Dr. Andrea Schüßler

Enc.:

Tokunaga et al. 1989 (MEDLINE-Abstract)

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30. April 2004

Aktenzeichen: PCT/EP02/14511
Anmelder: Petzelt
Unser Zeichen: P 3098 - sch / tz

In Response to the written opinion pursuant to Rule 66 PCT dated 03.02.2004

1. Claims

An amended set of claims 1 to 11 is filed herewith.

Claims 1, 6 and 8 are amended.
Claim 10 is cancelled and previous claims 11 and 12 are renumbered to claims 10 and 11, whereas the references to claim 10 are removed.

In Claim 1 the phrase "or a protein exhibiting biological properties thereof" is cancelled. The amino acid sequence of (a) is now identified as "the sequence marked with L" of the Figure 2(a) which is supported by the legend of Figure 2(a) on page 4 of the description which defines the "upper sequence" as the sequence of cyplasin-L. Further the sequence identifiers of the sequences of (a) and (b) in claim 1 are added in brackets.

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In claim 6 the phrase "exhibiting biological properties of cyplasin" is now removed from the claim and the protein now solely refers to the nucleic acid molecule of claim 1.

In claim 8 the protein obtained by the method is now further defined as being a "cytotoxic protein" which is supported on page 13, line 7 of the description.

2. Claim 8

The Examiner has objected that claim 8 is not sufficiently supported by the description and not clear.

However, it is described on page 13 of the description that a protein which is only cytotoxic for its host cell, if it reacts with the outside of the cell membrane of the host cell, can be produced by mammalian cells if its export from the host cells is blocked after synthesis. This general principle is conclusive and plausible. The Examiner did not give any concrete or substantiated ground why this principle of the invention cannot be performed over the whole range claimed. The claimed range clearly corresponds with the technical contribution to the art made by the applicants. It is noted that claim 8 solely relates to bioactive, i.e. cytotoxic, proteins. Thus, proteins, which are not cytotoxic after the harvest by cell lysis or homogenisation, are not encompassed by claim 8. The fact that the application does not contain any other example for a cytotoxic protein than cyplasin is no reason that the claimed method would not be reproducible for another protein which is only cytotoxic if reacting with the outside of the cell. According to the practice of the EPO an invention is sufficiently disclosed if at least one way is clearly indicated enabling the skilled person to carry out the invention. As the invention is reproducible over the whole claimed range, claim 8 fulfils the requirement of Article 6 PCT.

Further, the protein is not defined by the result to be achieved. Claim 8 is clearly characterised by the process steps (a) and (b), which solve the underlying techni-

cal problem. The expression "which is cytotoxic for said cells when secreted from said cells or externally applied" is a definition for the type of proteins produced by the claimed process, but is not a feature which is given to the proteins by the process of claim 8. On the opposite this feature refers to the natural mechanism of action of this type of proteins independently from their kind of production. As the method of claim 8 is clearly defined by the process steps (a) and (b), claim 8 fulfils the requirement of Article 6 PCT.

3. Unitiy of the invention

The claims comply with the requirement of unity according to Rule 13 PCT.

D3 does not disclose the recombinant expression of polypeptides devoid of their signal sequences which exhibit a cytotoxicity for their host cells. On page 74, right column, 2nd paragraph the authors of D3 report that "*deletion of the signal peptid region resulted in the loss of lethality*". From this result the authors further conclude that "*entering the secretory pathway is important for the toxicity*". Thus, D3 teaches away from the present application, because it describes that the secretory pathway is mandatory for the cytotoxicity. It is not derivable from D3 that proteins, which are cytotoxic if they are externally applied, still exhibit a cytotoxicity for their host cells if they are expressed without their leader sequence as inclusion bodies within the cell.

4. Novelty and inventive step

Claim 1 is now limited to cyplasin variants lacking their signal sequence. It is noted the a cleavage site for the signal sequence was found between aa positions 19 and 20 (page 34, Example 11) and the construct of Example 1 having a deletion from aa position 1 to 53 has to be only considered as an example of the invention. Thus, the amino acid sequence from position 20 to 558 of Figure 2 is claimed.

As the prior art does not indicate that that the claimed modified cyplasin can be produced in eukaryotic cells, whereas its cytotoxicity is maintained, the amended claims are novel and involve an inventive step.

European Patent Attorney

Dr. Andrea Schüßler

Enclosures:
Amended set of claims 1 to 11 (marked up version and clean copy)

Claims

1. An isolated nucleic acid molecule encoding the protein cyplasin with a deleted or non-functional secretory signal sequence, being selected from the group consisting of

(a) a nucleic acid molecule encoding a protein comprising the amino acid sequence from position 20 or 53 to position 558 of the sequence marked with "L" of Figure 2(a) (SEQ ID NO:1);

(b) a nucleic acid molecule comprising the sequence of Figure 2(b) (SEQ ID NO:5);

(c) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) or (b) due to the degeneration of the genetic code; and

(d) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a), (b) or (c).

2. A recombinant vector containing a nucleic acid molecule of claim 1.

3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.

4. A recombinant host cell which contains the recombinant vector of claim 2 or 3.

5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.

6. An isolated protein encoded by the nucleic acid molecule of

claim 1.

7. A method of making a protein exhibiting biological properties of cyplasin comprising:

(a) culturing the recombinant host cell of claim 4 under conditions such that said protein is expressed; and

(b) recovering said protein.

8. A method of making a cytotoxic protein in eukaryotic host cells which is cytotoxic for said cells when secreted from said cells or externally applied comprising:

(a) culturing a host cell transfected with a nucleic acid sequence encoding said protein with a deleted or non-functional secretory signal sequence under conditions such that said protein is expressed; and

(b) recovering said protein.

9. The method of claim 8 wherein the eukaryotic cells are mammalian cells.

10. A pharmaceutical composition comprising a nucleic acid molecule of claim 1 or a protein of claim 6.

11. Use of a nucleic acid molecule of 1 or a protein of claim 6 for preparing a pharmaceutical composition for treating cancer.

Claims

1. An isolated nucleic acid molecule encoding the protein cyplasin with a deleted or non-functional secretory signal sequence ~~or a protein exhibiting biological properties thereof~~, being selected from the group consisting of

(a) a nucleic acid molecule encoding a protein comprising the amino acid sequence from position 20 or 53 to position 558 of the sequence marked with "L" of Figure 2(a) (SEQ ID NO:1);

(b) a nucleic acid molecule comprising the sequence of Figure 2(b) (SEQ ID NO:5);

(c) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) or (b) due to the degeneration of the genetic code; and

(d) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a), (b) or (c).

2. A recombinant vector containing a nucleic acid molecule of claim 1.

3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.

4. A recombinant host cell which contains the recombinant vector of claim 2 or 3.

5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.

6. An isolated protein ~~exhibiting biological properties of cyplasin~~ encoded by ~~a~~ the nucleic acid molecule of claim 1.

7. A method of making a protein exhibiting biological properties of cyplasin comprising:

(a) culturing the recombinant host cell of claim 4 under conditions such that said protein is expressed; and

(b) recovering said protein.

8. A method of making a cytotoxic protein in eukaryotic host cells which is cytotoxic for said cells when secreted from said cells or externally applied comprising:

(a) culturing a host cell transfected with a nucleic acid sequence encoding said protein with a deleted or non-functional secretory signal sequence under conditions such that said protein is expressed; and

(b) recovering said protein.

9. The method of claim 8 wherein the eukaryotic cells are mammalian cells.

~~10. The protein produced by the method of claim 7 or 8.~~

10~~1~~. A pharmaceutical composition comprising a nucleic acid molecule of claim 1 or a protein of claim 6 ~~or 10~~.

11~~12~~. Use of a nucleic acid molecule of 1 or a protein of claim 6 ~~or 10~~ for preparing a pharmaceutical composition for treating cancer.